

**OBSERVATIONAL AND TRANSLATIONAL MULTICENTRE STUDY OF CANCER
PATIENTS INFECTED BY COVID-19: A EUROPEAN STUDY BY EURACAN NETWORK
(SARCOVID)**

Code: SARCOVID

Sponsor: CENTRE ANTICANCEREUX LEON BERARD (CLB), Lyon, France

Principal Investigator: Dr. Javier Martín Broto (H. Virgen del Rocío, Seville, Spain)

Protocol version and date: V 1, 29 April 2020

SUMMARY

Sponsor	EUropean Rare Adult solid CAncer Network (EURACAN)
Study title	OBSERVATIONAL AND TRANSLATIONAL MULTICENTRE STUDY OF CANCER PATIENTS INFECTED BY COVID-19: A EUROPEAN STUDY BY EURACAN NETWORK (SARCOVID)
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Duration	1 year

Study design	<p>Post-authorisation, multicentric, observational, retrospective and prospective study.</p> <p>Data registration period: March-December 2020 to compare with March-December 2019</p>
Study Objectives	<p>1. On one hand, to identify the proportion and profile of cancer patients that have been indirectly affected by COVID-19 outbreak. Thus, we propose to analyze those cases that have suffered any delay, cancellation, or relevant change of any active treatment against their tumors whatever the aim of them. A secondary objective is to analyze the impact of the COVID-19 outbreak on the management of sarcoma patients in European reference centres from EURACAN.</p> <p>2. On the other hand, to collect cancer patients that have been directly affected by COVID-19 infection in order to analyze their evolution through the infection and to correlate the clinical outcome with circulating cytokines.</p>
Samples to be used	Plasma samples for translational study
Inclusion criteria	<ul style="list-style-type: none"> • Cancer patients (preferentially sarcoma patients) and cancer patients infected by COVID-19. • ≥ 18 years • Available clinical and treatment information
Number of patients	<p>For the observational study, no estimation is made. Cancer patients included in the EURACAN network, all who sign the IC and who meet the study criteria.</p> <p>For the translational 20 cancer patients vs 20 non-cancer.</p>

Rationale

Patients with cancer constitute a large, immunosuppressed population. Cancer immunoediting, which represents the interplay between tumor and immune system, leads ultimately to changes in immune cells, immune modulators, cytokines, and molecules expression towards the escape phase and the development of an immunosuppressive tumor microenvironment.¹ Chemotherapy, surgical resection, and newer treatments compromising immune function, make cancer patients more susceptible to infections.²

The immune system plays a key role in the COVID-19 evolution. COVID-19 causes overwhelming persistent innate-induced inflammation that can lead to a cytokine storm, cytokine-associated lung injury, and diffuse organ involvement.³ Alterations of the CD4⁺ and CD8⁺ T cells subsets have been observed, with loss of functional diversity in CD4⁺ T cells, and increased expression of regulatory molecules in CD8⁺ T cells.^{4, 5} Hence, it can be assumed that the systemic immunosuppressive state of cancer patients might result in an increased risk of COVID-19 infection and poorer prognosis for this group of patients.

In severe COVID-19 acute respiratory distress elevated interleukin (IL)-6 seems to be the hallmark inflammatory signature seen in serum of patients,⁶ thus the use of IL-6 or IL-6-receptor blocking antibodies have been encouraged. This cytokine is able to block CD8⁺ cytotoxic T-cells by inhibiting the secretion of INF- γ , induce suppression of cytokine signaling and paralyze the cell-mediated antiviral response causing an increase of PD-1 expression.⁷

We clearly believe that there is a need of studies focusing on particular groups, as the case of oncologic patients, to whom it must be given immediate consideration, and recommendations addressed for the use of the newly proposed therapeutic approaches for COVID-infected patients. Until now, very few observations have been made about COVID-19 infection in patients with cancer. Epidemiological statistics of the cases in China showed that oncologic patients comprised of 1% (18 out of 1590) of COVID-19 infected patients, while it is estimated that oncologic patients represent 0.29% of the general population in China. Thus, a higher percentage of oncologic patients in the COVID-19 cohort than in the overall population.⁸ Even if this does not allow to draw conclusions given the small sample size and heterogeneity of cancer types considered in the study, in addition to other variables such as disease course and diverse treatment strategies, patients with cancer were observed to have a higher risk to become in severe condition or died than other COVID-19

infected. More specifically, 7 over 18 (39%) oncologic patients infected by COVID-19 required invasive ventilation, whereas this was required in 124 over 1572 (8%) of non-neoplastic patients with COVID-19 infection (Fisher's exact $p=0.0003$).⁸

Therefore, it appears that in at-risk populations, such as cancer patients, with greater probability that an adequate adaptive immune response induced by the innate immune system fails, the persistent self-induced inflammation can cause mortality.⁹ So far, several studies have focused on the description of the cytokine expression profile in patients during COVID infection, each in a relatively small group of infected patients. The most common laboratory abnormalities observed were depressed total lymphocytes, prolonged prothrombin time and elevated lactate dehydrogenase. Compared with non-ICU patients, patients who received ICU care had numerous laboratory abnormalities, suggesting that COVID-19 infection may be associated with cellular immune deficiency, coagulation activation, myocardia injury, hepatic injury, and kidney injury. Neutrophilia may be related to "cytokine storm" induced by virus invasion, coagulation activation could have been related to sustained inflammatory response, and acute kidney injury could have been related to direct effects of the virus, hypoxia, and shock.^{6, 10}

Considering cytokines, COVID-19 infected patients plasma showed high amounts of IL-6, IL-1 β , IFN- γ , interferon gamma-induced protein 10 (IP-10), and monocyte chemoattractant protein-1 (MCP-1), probably leading to activated T-helper-1 (Th1) cell responses. Moreover, patients requiring ICU admission exhibited higher concentrations of granulocyte-colony stimulating factor (G-CSF), IP-10, MCP-1, macrophage inflammatory protein 1- α (MIP-1 α), and tumor necrosis factor- α (TNF- α) than those not requiring ICU admission, suggesting that the cytokine storm was associated with disease severity. However, infection also initiated increased secretion of T-helper-2 (Th2) cytokines (eg, IL-4 and IL-10) that suppress inflammation. Further studies are necessary to characterise the Th1 and Th2 responses in COVID-19 infection and to elucidate the pathogenesis.^{4, 11}

Remarkably, COVID-19 illness exhibits three grades of increasing severity, which correspond with distinct clinical findings, response to therapy and clinical outcome:

- Stage I mild - Early Infection (3–7 days). SARS-CoV-2 binds to its target using the angiotensin-converting enzyme 2 (ACE2) receptor on human cells. These receptors are abundantly present on human lung and small intestine epithelium, as well as the vascular endothelium. As a result,

the infection usually presents with mild respiratory and systemic symptoms. Peripheral blood leucocytes and lymphocytes are not significantly reduced at this phase.

- Stage II - moderate (7–14 days after the onset of the symptoms). Viral multiplication and localized inflammation in the lung. Markers of systemic inflammation may be elevated, but not remarkably.
- Stage III – severe. Patients have an extra-pulmonary systemic hyperinflammation syndrome. In this stage, markers of systemic inflammation appear to be elevated. Plasma proportion of CD4-positive T lymphocytes, CD8-positive T lymphocytes, and B lymphocytes significantly decreases. Studies have shown that inflammatory cytokines and biomarkers such as IL-2, IL-6, IL-7, GCSF, MIP-1 α , TNF- α , C-reactive protein, ferritin, and D-dimer are significantly elevated in those patients with more severe disease. Troponin and N-terminal pro B-type natriuretic peptide (NT-proBNP) can also be elevated.^{12, 13}

To the best of our knowledge, studies aiming to characterize the effect of coronavirus on the production of cytokines and chemokines in the oncologic patient cohort are still lacking. What does it happen, when the overexpressed immunosuppressive cytokines, the enhanced functional immunosuppressive leukocyte populations, impaired dendritic cell maturation and suppressed induction of proinflammatory danger signals that describe cancer status, are the starting point of the coronavirus infection? From these issues and considerations derives the urgent need to focus and design a study targeted at cancer patients infected by COVID-19.

In this COVID-19 outbreak, the major risk for patients with cancer is the inability to receive necessary medical services but also nosocomial infections. Decisions on whether or not to postpone cancer treatment and clinical trials need to be made on a patient by patient basis, and according to the risk for the patient and the prevailing situation because delays could lead to tumour progression and ultimately poorer outcomes.¹⁴ Beyond the increase of the risk of requiring mechanical ventilation and death that should be prospectively analyzed in oncologic patients with COVID infection, it is also relevant to study the impact of the precautionary principle that is implemented in our patients.¹⁵ This latter implies that cancer patients have suffered delays or cancellation of surgeries, on radiation therapies or systemic treatments. In the context of rare cancers, the access to experts could be an additional challenge while the majority of European citizens are confined at home for weeks.

In line with our commitment to promote cancer patients care, and working within the European Rare Adult solid CAncer Network (EURACAN), we aim to carry out a descriptive and prospective observational study considering the impact of COVID-19 outbreak in two different cancer subpopulations.

EURACAN is the European Reference Network dedicated to rare adult solid cancers. The network gathers 66 health care providers in 17 European countries, identified based on documented expertise, experience treating rare cancers, and endorsement by their member state. It groups all rare solid adult cancers into ten “domains” corresponding to the RARECARE list of rare cancers based on the International Classification of Diseases for Oncology (ICD-O): domain 1 is dedicated to sarcomas.

The European Society for Medical Oncology (ESMO) and EURACAN have published Clinical Practice Guidelines for bone sarcomas, soft tissue and visceral sarcomas and GIST in 2019.

Objectives

- 1.- On one hand, to identify the proportion and profile of cancer patients that have been indirectly affected by COVID-19 outbreak. Thus, we propose to analyze those cases that have suffered any delay, cancellation, or relevant change of any active treatment against their tumors whatever the aim of them. A secondary objective is to analyze the impact of the COVID-19 outbreak on the management of sarcoma patients in European reference centres from EURACAN.
- 2.- On the other hand, to collect cancer patients that have been directly affected by COVID-19 infection in order to analyze their evolution through the infection and to correlate the clinical outcome with circulating cytokines.

Methodology

- 1.- Retrospective and prospective registry, to collect the clinical information of patients treated in hospitals within the EURACAN network. The format for ethic committees will be non-interventional observational study.
- 2.- A web-based registry will be designed to collect clinical data in an anonymous manner. In detail, data fields will include the following information:

- DATA CONCERNING NEOPLASTIC CONDITION: demographics (gender, date of birth), specific dates (appearance of first symptom or sign, performance of biopsy, different treatments, recurrence, death, and last follow-up), diagnosis (imaging tests for primary tumor location and metastasis workup, cancer histology, grade, size), therapeutic approach (type of surgical resection, perioperative treatments, systemic treatments for metastatic recurrence).
- DATA CONCERNING THE IMPACT OF COVID-19 OUTBREAK IN ONCOLOGIC MANAGEMENT: Data of any delay or cancellation in any of the following procedures: diagnostic workout (imaging and biopsy), surgery, radiation therapy, systemic treatment, imaging test in follow-up and surveillance. Comparison between March-December 2020 and March-December 2019 periods and comparison with guidelines.
- DATA REGARDING COVID-19 INFECTION: date of COVID infection, hospital admission requirement, ICU admission requirement, mechanical ventilation (invasive or non-invasive) requirement, date of discharge, clinical evolution. Evolution and modification in the treatment plan if any.

A query-based task will be used for remote data cleaning, and on-site monitoring will be carried out for leading recruiting centers.

For variables with binomial distributions, frequencies and percentages will be calculated with corresponding 95% CIs. To compare categorical variables, Fisher's exact or χ^2 tests will be used when applicable. ECOG status will be correlated with dose reductions or interruptions using χ^2 in a post-hoc analysis. Time-to-event variables (overall survival and progression-free survival) will be measured from the date of therapy onset and will be estimated using Kaplan-Meier survival analysis. Comparisons between the variables of interest will be made using the log-rank test.

Multivariate analyses with the variables that appeared to be significant in the univariate analysis will be done using the Cox proportional hazard regression model. The validity of proportional hazard assumption will be verified by plotting two log-log survival curves and adding a time-dependent variable to each model to confirm that the hazard ratio for each covariate did not increase or decrease over time. All p values reported will be two-sided, and statistical significance will be defined as a p value of less than 0.05. The software package used for the analysis will be SPSS Statistics (version 20).

Translational associated study

To evaluate the immune-response in plasma samples of patients with COVID-19 confirmed infection, plasma will be separated from peripheral blood and used to determine the expression of soluble proteins (i.e. cytokines, chemokines, etc...). Protein expression will be quantified using ProcartaPlex immunoassays, more precisely the Immune Monitoring 65-Plex Human Panel (ThermoFisher Scientific; Waltham, MA, USA). For differential expression analysis, blood samples will be collected prospectively at the admission time, at day 8 and 14.

Subject protection

1. Evaluation risk-benefit for subjects in investigation

This study will not have any prejudice on the patients included as it is an observational study and no intervention or procedure is mandatory by protocol. The possible benefit is obtained during the prospective part of the trial after sites have improved their quality systems and patient illness management.

The medication is assigned individually by each investigator following the standard clinical practice of each participant site.

2. Informed Consent

All patients should sign and date the Informed Consent Form after reading the patient Information Sheet to accept participating in this study.

Participating in the study is voluntary and the patient can withdraw his/her consent at any time, without giving any reason and without reducing his/her right to health care. He/she can also withdraw the consent to use the sample donated to the study without withdrawing the participation in the study (the patient's clinical data will be registered and analysed). Any withdraw should be confirmed by signing a Revocation Form.

However, the investigator should try to know the reason to withdraw consent in order to improve the study conditions.

3. Procedure of Informed Consent

Usually the patient, or in case of disability or death, his/her relatives or legal representatives, must give his/her written consent before entering the observational study. This document includes the consent for donation of an archive sample for the translational sub-studies.

To obtain consent, the investigator must meet physically with the patient, and/or relative or legal representative and explain the nature, purpose, possible benefits and consequences of the study in a comprehensible manner, so he/she can accept or decline freely his/her participation. The informed consent form will be given to the patient with enough time for him/her to read it and evaluate it. The investigator must answer any doubts that the subjects, relatives or legal representative may have.

The patient must comprehend that he/she has the right to withdraw from the study at any time without any reason and without his/her care being affected by it.

The investigator must not start any activity from the study until he/she has obtained the patient's informed consent. The investigator should write in the clinical history that the Principal Investigator or a sub-investigator has explained the patient the study and has solved any question and the patient accepts participating in the study.

Taking into account the retrospectivity of part of the study, it may occur that patient has died and is not able to sign the consent. The investigator must then ensure there are no previous dispositions from the patient opposing to the use of his/her clinical data or. The investigator should do an effort to contact the relatives to ask for permission to collect clinical data. If this effort was unsuccessful, the PI should follow national law regarding the possibility of collecting this data in the absence of consent. If collection is possible an Absence of Consent Form should be completed and signed.

The data that is obtained this way will follow the same safety regime and data protection as the data collected after signature of informed consent form.

4. Data protection

In order to ensure confidentiality, this study should follow at least European data protection regulation (RGDP 2016/679) on the protection of natural persons with regard to the processing of personal data and on the free movement of such data.

The data collected in this study will be only accessible for the trial Sponsor and its designees, for monitoring/auditing procedures, the site investigator and collaborators, the Ethics Committee of each corresponding site and the Health Authorities.

All information, oral or written, even unpublished information that is handed to the Investigators, including protocol and CRFs, must be considered as Sponsor property.

All data or any material from the study cannot be disclosed by the Investigator and/or collaborator to any unauthorized third party, without written consent of the Sponsor. Investigator and the Institution will allow access to data and source documentation for monitoring, auditing, Ethic Committee revision and inspections of Health Authority, but maintaining at all times subject personal data confidentiality. The Investigator must guarantee that patient anonymity is kept at all times and their identity must be protected from unauthorized persons and institutions. All patients included in the study will be identified with a numeric code, so that no identifiable personal data will be collected on the Sponsor database (except for date of birth, what is specifically indicated in the IC).

The Investigator must have and conserve a patients' inclusion registry where the next personal data of the patient is registered: name, surname, gender, date of birth and the corresponding identification code in the study. This information could be done with the site usual registration system or with a form provided by the Sponsor. In any way, the information collected should not be shared outside the participating site.

Other considerations

1. Protocol amendments

The study will be performed following the study protocol. The investigator must not make any changes to the protocol without prior consent of the sponsor and approval of the corresponding Ethics Committee, if applies.

Any amendments for this study protocol must only be performed by the Sponsor. The original protocol and any amendments must be signed by the principal investigator in the Protocol Signature page.

Every amendment or administrative changes will be incorporated to the actual version of the protocol and distributed to all Investigators and collaborators.

Protocol deviation refers to any procedure that it is not contemplated in the protocol but does not affect to patient's consent of participation, the inclusion/exclusion criteria and/or good clinical practice. A deviation is a minor event that has no impact in the study or the patient. All deviations must be communicated to the sponsor within 5 days since it took place.

A protocol violation is any procedure that it is not contemplated in the protocol and does affect to patient's consent of participation and/or inclusion/exclusion criteria and/or good clinical practice. A violation is considered a major event because have an impact in the study. The violations must be communicated to the sponsor within 24 hours since it took place. In this case, the sponsor will decide if the patient data can be included in the database.

2. Study Documents Maintenance

The Investigator must conserve appropriately and secure all clinical trial file, as well as all data for following verification. These documents must be classified in two different categories: 1) Investigator Site File 2) Patient's source documents.

The Investigator Site File must contain the protocol and all amendments, Ethic Committee approval, authorization of Health Authorities, the Informed Consent document, curriculum vitae of all site personnel involved in the trial, inclusion registry, guidelines and any other important or relevant documentation.

The patient source documents are: patient medical nursing and pharmacy preparation / dispensing records, original laboratory reports, tests reports, radiology, pathology or any other procedures reports. The Informed Consent signed by the patient is also considered as source documents.

The Investigator must keep this files stored according to national legislation. Once this time has elapsed, the documentation can be destroyed, depending on country own laws and administrative regulations. If the Investigator wants to delegate custody of this documents to a third party or store them in a different place than the agreed, it must be notified to the Sponsor.

3. Auditing and inspections

All data collected from patients in the eCRF must be truthful and conformant to source data.

To ensure good clinical practice when collecting data, the sponsor or regulatory authorities can request an auditing or inspection from one or more sites participating in the study. This auditing/inspection will be notified to involved parties with at least 4 weeks in advance. The investigators will compromise to facilitate the auditors/inspectors work by providing all patient's source documents included in the study as well as the investigator files, access to internet and eCRF.

4. Ethics

This study will be performed according to the Ethic Principles originated in The Declaration of Helsinki adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975; 35th WMA General Assembly, Venice, Italy, October 1983; 41st WMA General Assembly, Hong Kong, China, September 1989; 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996, and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000, clarification note of paragraph 29, added by WMA, Washington 2002, Clarification note of paragraph 30, added by WMA General Assembly, Tokyo, Japan, 2004, 59th General Assembly, Seúl, Korea, October 2008 (Annex XII), 64th General Assembly, Fortaleza, Brazil, (October 2013) and the Good Clinical Practice issued by the work group of Efficacy of Medicinal Substances of the European Community (1990) (CPMP/ICH/135/95) and all legal requirements and laws of each country participating in the Clinical Trial.

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